

IN THE CLAIMS:

The following Listing of Claims replaces all prior Listings and versions of claims in this application.

Listing of Claims

1-39. (Cancelled)

40. (Currently Amended) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

i) transformation of said microorganism with a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase, wherein said synthase ~~which~~ has glucosamine-6-phosphate synthase activity; and

ii) genetic modification of the coding region of a gene encoding glucosamine-6-phosphate synthase ~~that increases the activity of said glucosamine-6-phosphate synthase~~, wherein said genetic modification results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said coding region of said gene ~~encoding glucosamine-6-phosphate synthase~~, wherein said at least one nucleotide modification ~~resulting results~~ in increased glucosamine-6-phosphate synthase activity;

wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b) recovering and purifying said product.

41. (Previously Presented) The method of Claim 40, wherein said glucosamine-6-phosphate is intracellular and said glucosamine is extracellular, wherein said step of recovering comprises a recovering step selected from the group consisting of recovering said

glucosamine-6-phosphate from said microorganism, recovering said glucosamine from said fermentation medium, and a combination thereof.

42. (Previously Presented) The method of Claim 40, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering comprises isolating said glucosamine-6-phosphate from said microorganism.

43. (Previously Presented) The method of Claim 40, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering further comprises dephosphorylating said glucosamine-6-phosphate to produce glucosamine.

44. (Previously Presented) The method of Claim 40, wherein said step of culturing comprises maintaining said source of carbon at a concentration of from about 0.5% to about 5% in said fermentation medium.

45. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed at a temperature from about 30°C to about 40°C.

46. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed at about 30°C.

47. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed in a fermentor.

48. (Previously Presented) The method of Claim 47, wherein said step of culturing is performed under conditions wherein glucose is added to said fermentation medium at a rate in which glucose accumulation in said fermentation medium is undetectable.

49. (Previously Presented) The method of Claim 47, wherein said step of culturing is performed so that an excess of glucose is maintained.

50. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 21 mg/L of said product.

51. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 1 g/L of said product.

52. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 5 g/L of said product.

53. (Previously Presented) The method of Claim 40, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase enzymatic activity, wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence.

54. (Previously Presented) The method of Claim 53, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

55. (Previously Presented) The method of Claim 53, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase comprises a genetic modification which increases the activity of said glucosamine-6-phosphate synthase.

56. (Previously Presented) The method of Claim 40, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase or said gene encoding said glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase.

57. (Previously Presented) The method of Claim 40, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme  $\Pi^{\text{Nag}}$ , phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme  $\Pi^{\text{Glc}}$  of the PEP:glucose PTS, and EIIM,P/III<sup>Man</sup> of the PEP:mannose PTS, wherein said genetic modification decreases the activity of said protein.

58. (Previously Presented) The method of Claim 40, wherein said microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the activity of said phosphatase.

59. (Previously Presented) The method of Claim 40, wherein said microorganism has additional modifications in genes encoding the following proteins: *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase and *N*-acetyl-glucosamine-specific enzyme  $\Pi^{\text{Nag}}$ ;

wherein said genetic modification decreases the activity of said proteins.

60. (Previously Presented) The method of Claim 40, wherein said microorganism has additional modifications in genes encoding *N*-acetylglucosamine-6-phosphate deacetylase and glucosamine-6-phosphate deaminase;

wherein said genetic modification decreases the activity of said proteins.

61. (Previously Presented) The method of Claim 60, wherein said genetic modification is a deletion of at least a portion of said genes.

62. (Previously Presented) The method of Claim 40, wherein said microorganism is selected from the group consisting of bacteria and yeast.

63. (Previously Presented) The method of Claim 40, wherein said microorganism is a bacterium of the genus *Escherichia*.

64. (Previously Presented) The method of Claim 40, wherein said microorganism is *Escherichia coli*.

65. (Previously Presented) The method of Claim 64, wherein said microorganism comprises at least one additional genetic modification which is a mutation in an *Escherichia coli* gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glmS*, *ptsG* and a phosphatase gene, wherein said genetic modification decreases the activity of a protein encoded by said gene.

66. (Previously Presented) The method of Claim 40, wherein said microorganism is a yeast.

67. (Currently Amended) A recombinant microorganism for producing glucosamine by a biosynthetic process, said microorganism being transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding glucosamine-6-phosphate synthase, said nucleic acid sequence being operatively linked to a transcription control sequence and comprising, in the coding region of the nucleic acid sequence, a genetic modification which increases the activity of said glucosamine-6-phosphate synthase;

wherein expression of said nucleic acid sequence increases production of glucosamine by said microorganism.

68. (Currently Amended) The recombinant microorganism of Claim 67, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme  $\Pi^{\text{Nag}}$ , phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme  $\Pi^{\text{Glc}}$  of the PEP:glucose PTS, and EIIM,P/III<sup>Man</sup> of the PEP:mannose PTS, wherein said genetic modification decreases the activity of said protein.

69. (Currently Amended) The recombinant microorganism of Claim 67, wherein said microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the activity of said phosphatase.

70. (Previously Presented) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism having at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase wherein said genetic modification is selected from the group consisting of:

i) transformation of said microorganism with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase activity; and

ii) genetic modification of a gene encoding glucosamine-6-phosphate synthase that increases the activity of said glucosamine-6-phosphate synthase, and wherein said genetically modified microorganism is produced by a process comprising the steps of:

(1) generating modifications in an isolated nucleic acid molecule comprising a nucleic acid sequence encoding glucosamine-6-phosphate synthase to create a plurality of modified nucleic acid sequences;

(2) transforming microorganisms with said modified nucleic acid sequences to produce genetically modified microorganisms;

(3) screening said genetically modified microorganisms for glucosamine-6-phosphate synthase activity; and,

(4) selecting said genetically modified microorganisms which have increased glucosamine-6-phosphate synthase activity;

wherein said step of culturing produces a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and,

b) recovering said product.

71. (New) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism that has been transformed with a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase, wherein said synthase has glucosamine-6-phosphate synthase enzymatic activity, and wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b) recovering and purifying said product.

72. (New) The method of Claim 71, wherein said glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase.

73. (New) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

i) transformation of said microorganism with a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase from a

microorganism, wherein said synthase has glucosamine-6-phosphate synthase activity; and

ii) genetic modification of the coding region of a gene encoding glucosamine-6-phosphate synthase, wherein said genetic modification results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said coding region of said gene, wherein said at least one nucleotide modification results in increased glucosamine-6-phosphate synthase activity;

wherein said step of culturing produces and accumulates at least about 21 mg/L of a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b) recovering and purifying said product.

74. (New) The method of Claim 40, wherein the glucosamine-6-phosphate synthase is from a microorganism.

75. (New) The method of Claim 40, wherein the glucosamine-6-phosphate synthase is from a bacterium.

76. (New) The method of Claim 40, wherein said product is extracellular glucosamine-6-phosphate and said step of recovering comprises recovering said glucosamine-6-phosphate from said fermentation medium.